Effects of Loading Density and Transport Water Volume on Ammonia Production, Stress, and Survival of Sacramento-San Joaquin Delta Fishes

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Summary

Many resident and transient species of fish to California's Sacramento-San Joaquin Delta (SSJD) have experienced precipitous declines in abundance over the last half century (Bennett and Moyle 1996, Moyle 2002, Brown and Moyle 2005). Though there are likely a multitude of factors that have contributed to the current state of the SSJD fishery (Moyle 2002), direct and indirect effects of southern SSJD water diversion facilities are commonly cited (Moyle and Williams 1989, Arthur *et al.* 1996, Brown *et al.* 1996). One particular indirect effect of SSJD water diversion facilities are stressors associated with transporting fish by truck away from state and federal owned fish collection facilities, for subsequent release at northern SSJD sites, as a means to prevent fish entrainment and pump induced mortality. Between 2000 and 2003 such operations resulted in the transportation of an average of nearly seven million fish per year (USBR 2009), including native species of concern, like endangered delta smelt (*Hypomesus transpacificus*) and Chinook salmon (*Onchorhynchus tschawyscha*), and ecologically important non-native Pelagic Organism Decline species, like threadfin shad (*Dorosoma petenense*) and striped bass (*Morone saxatilis*). Such operations may ultimately have population level effects on some species, and continued improvement of fish collection, handling (loading), transport, and release operations are a priority for both state and federal water resource agencies.

Fish-transport from SSJD fish collection facilities consists of hauling fish in a closed (*e.g.*, no additional water provided throughout transport) cylindrical tank (1.2-m-deep, 4.4-m-long, mean volume post-transport = 6,455 L) that is provided continuous pure O_2 via oxygen diffusing airstones, over a maximum distance of 49.9 km (Sutphin and Wu 2008). To maintain fish health and maximize long-term survival, stressors that are common during such operations, including handling, confinement, unfavorable densities, and degraded water quality conditions, must be considered (Piper *et al.* 1982, Berka 1986, Sutphin and Wu 2008). Maintenance of appropriate water quality conditions is often the limiting factor during fish transport, and is generally considered when developing fish transportation tables (Berka 1986, Emata 2000). In 2006 Reclamation biologists initiated a multi-phase research program to develop density and temperature dependent fish transportation tables, as a function of oxygen consumption and total ammonia nitrogen (TAN) production of SSJD fishes, for use at SSJD fish collection facilities. However, it is possible, particularly during short durations (<2 h), densities that permit appropriate water quality conditions are high enough to expose fish to physical (*i.e.*, abrasions and scale loss) and physiological stress (*i.e.*, crowding) that will effect long-term survival. Density induced stress and associated nonspecific interactions may also affect fish metabolism, resulting in increased oxygen consumption and ammonia production (M_{TAN}) rates of transported fish. Fish transport truck oxygen production systems can generally be adjusted to meet the oxygen consumption demands of high densities of fish (B. Bridges 2009, personal communication). However, in closed (no additional water added) transport systems accumulated excretory products of fish can result in elevated levels of TAN and unionized ammonia which can impair performance, health and survival of fish (Meade 1985, Russo and Thurston 1991). Ammonia production may be exacerbated when fish are transported at high densities as a result of stress induced increases in metabolic rates. Measuring density dependent M_{TAN}, physiological stress and chronic (96-h) mortality of transported fish, paired with current research (water quality derived fish transportation tables), will provide information on methods for minimizing stress endured and maximizing acute and chronic fish survival during fish transportation operations from SSJD fish collection facilities.

Problem Statement

Fish transportation tables currently being re-developed by Reclamation biologists for use at south SSJD fish collection facilities are intended to provide fish diversion workers with the maximum temperature dependent density of fish that can be maintained for approximately 60–70 min to assure that unhealthy levels of ammonia (TAN and unionized ammonia) and oxygen are not reached. However, because transport operations from SSJD fish collection facilities are short in duration (<2 h) it is possible that densities recommended by the updated fish transportation tables may be high enough to expose fish to physical and physiological stress that will impair health and survival. Measuring density dependent M_{TAN}, physiological stress and chronic (96-h) mortality, paired with current Reclamation research (water quality derived fish transportation tables), will provide information on methods for minimizing stress endured and maximizing acute and chronic fish survival during fish transportation operations from SSJD fish collection facilities.

Goals and Hypotheses

Goals:

1. Determine if additional physiological stress is caused by transporting fish at elevated densities (grams of fish/liter of water) and reduced volume (75% of full) of water, and if there is an optimal density at which fish should be transported to minimize physiological stress.

2. Determine if transporting fish at varying densities and reduced volume of water (75% of full) affect post-transportation mortality (96-h) and if there is an optimal density at which fish should be transported to minimize post-transport mortality.

3. Determine if transporting fish at varying densities and reduced volume of water (75% of full) affect metabolic rates of fish, as a function of M_{TAN}, and if there is an optimal density at which fish should be transported to maintain appropriate M_{TAN} levels so TAN levels do not exceed 2 mg/L during transport.

Hypotheses (Null):

1. There will be no difference in blood constituent levels (Hct., glucose, lactate, cortisol) of fish exposed to densities of 15, 30, 60, 120 and 240 g of fish/liter of water measured immediately after and 12-h post transport compared to basal levels (measured prior to transport).

2. There will be no difference in blood constituent levels (Hct., glucose, lactate, cortisol) between fish exposed to densities of 15, 30, 60, 120 and 240 g of fish/liter of water measured immediately after and 12-h post transport.

3. There will be no difference in blood constituent levels (Hct., glucose, lactate, cortisol) between fish exposed to 75 and 100% water volume during transport measured immediately after and 12-h post transport.

4. There will be no difference in acute (immediately following transport) and chronic survival (96-h post transport) between fish exposed to densities of 15, 30, 60, 120 and 240 g of fish/liter of water during transport.

5. There will be no difference in acute (immediately following transport) and chronic survival (96-h post transport) between fish exposed to 75 and 100% water volume during transport.

6. There will be no difference in M_{TAN} between fish exposed to densities of 15, 30, 60, 120 and 240 grams of fish/liter.

7. There will be no difference in M_{TAN} between fish exposed to 75 and 100% water volume during transport.

Materials and Methods

Source and Care of Fish

Threadfin shad and striped bass were selected for this study because (1) they are Pelagic Organism Decline species, (2) the two most abundant species salvaged at the SSJD fish collection facilities, and (3) are commonly present when high densities of fish are salvaged at the facilities. Adult threadfin shad and juvenile striped bass will be netted from a school of wild fish entrained at Reclamation's Tracy Fish Collection Facility (TFCF) in Byron, California, and held at the TAF in continuously aerated 757-L circular flow-through tanks. Water temperatures will be maintained at target temperature ± 0.5 °C (where initial target temperature will be the temperature at which they were salvaged) and fish will be maintained under a natural photoperiod (37° 44' 23" N). If changes in water temperature are required, rate of change will be < 1.0 °C/day. Fish will be fed an appropriate diet at 3% body weight per day. Prior to testing fish will randomly be isolated as a function of treatment condition (density × transport container water volume) into individual holding tanks and provided a unique mark using a fluorescent microsphere solution (New West Technologies, Santa Rosa, California). During this fish marking process a random sub-sample of 40 fish from each holding tank will be weighed (g), to provide predetermined estimates of experimental densities and to isolate each experimental treatment by holding tank prior to experimentation. Post marking, fish will be maintained at holding conditions for at least 2 weeks, during which mortality rates and feeding will be monitored to assure fish are healthy prior to experimentation. During this post-marking period, food rations will be reduced to 2% body weight per day to limit fish growth before testing.

Experimental Protocol: Effects of Elevated Densities on Stress and Survival of Sacramento-San Joaquin Delta Fishes Transported by Truck.

Test fish will be randomly designated to one of two treatment groups, (1) nearly 100% full transport containers or (2) 75% full transport containers (14.2-L of water in a 19-L bucket) and will be loaded, at densities of approximately 15, 30, 60, 120 and 240 g of fish/liter of water, into 19-L buckets using fine mesh dip nets. Test densities are based on those measured by Sutphin and Wu (2008) during standard fish transportation operations from the TFCF (0.3–64.5 g/L), those recommended by preliminary water quality derived fish transportation tables data (100–175 g/L) to maintain TAN levels below 2 mg/L, and those that could potentially be achieved when large schoals of fish are salvaged at the fish collection facilities (>200 g/L). Assuming adult threadfin shad and juvenile striped bass weigh approximately 10 g, our transport densities equivocate to 29, 57, 114, 228 and 456 fish per 19-L bucket. Pilot studies will be conducted to determine the suitability of densities >200 g/L. Two control fish will be removed from each holding tank prior to testing, transferred to a bath containing a lethal dose of tricaine methansulfonate (MS-222; Argent Chemical Laboratories, Inc.; 200 mg/L), and sampled for blood according to methods outlined in Portz (2007). Treatment fish will then be transported for 60 min on a flatbed truck and returned to the TAF. Two treatment fish will be immediately removed from individual fish transport container and bled in the identical manner as control fish. To simulate TFCF fish transportation operations, fish will be provided oxygen throughout transport, but no effort will be made to control other water quality parameters. After post-transport blood samples are collected two fish from

each treatment (density × water volume) will be transferred to individual 19-L buckets, which will be perforated and floated in 757-L holding tanks. Twelve hours after transport, these fish will be sampled for blood. The remaining treatment fish will be combined (using water to water transfer from transport tanks) into a single 757-L fish holding tanks for post transport evaluation of survival. Water temperature (°C), dissolved oxygen (mg/L), TAN (mg/L), carbon dioxide (CO_2, ppm), and pH levels will be measured before and after transport in each individual transport container, and daily in each holding tank. Again, assuming adult threadfin shad and juvenile striped bass weight approximately 10 g, this equivocates to 884 fish per tank and a density of 11.5 g of fish/L during post-transport mortality assessments.

Plasma Analysis

Blood samples will be immediately centrifuged for 4 min at 12,000 × g, effectively separating blood plasma from packed cells. Blood haematocrit (Hct.) levels for each individual sample will be recorded immediately and the plasma will be transferred to cryogenic freezing vials and stored in a liquid-nitrogen dewar flask. Plasma lactate and glucose concentrations will be measured with a polarographic analyzer (YSI 2700 Select, Yellow Springs, Inc., Yellow Springs, Ohio.) and plasma cortisol concentrations will be measured by the University of California Davis Endocrinology Laboratory using a modified enzyme immunoassay.

96-h Mortality Analysis

After transport fish will be monitored at 2, 6, 12, 24, 48, 72, and 96 h post transport. Dead fish will be removed, identified by mark, and measured for length (fork, standard and total lengths in mm) and wet weight (g). After 96 h is complete a sub-sample of 10 fish from each treatment will be measured for length and weight, and a group wet weight will be obtained as a function of treatment type.

Ammonia Production Rates (M_{TAN})

Density dependent (group-mediated) M_{TAN} of fish will be estimated for each treatment condition using the following equation:

$$M_{TAN}\ (mg/g/h) = (TAN_{t0} - TAN_{t1}) \times V \times TW$$

Where TAN_{t1} is the TAN level (mg TAN/L) before fish are inserted into the transport tanks, TAN_{t0} is the TAN level after transport, V is the transport tank volume minus the volume of the fish transported, and TW is the total weight (g) of the transported fish.

Sample Size and Estimate of Time Required for Completion

A power calculation was carried out using post stress 96 h fish survival data from Hasan and Bart (2007) and post stress fish plasma constituent data from Port (2007). Hasan and Bart (2007) assessed the effects of loading density (200, 300, and 400 g/L) and transport stress on mortality and physiological stress responses for rohu (*Labeo ronita*). Portz (2007) measured the effects of handling stress on plasma constituent levels of juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Based on immediate and

delayed mean mortality of transported fish at densities of 200, 300, and 400 g/L, as reported by Hasan and Bart (2007), and mean plasma constituent levels before and after 1 h transport and before and after handling stress as reported by Hasan and Bart (2007) and Portz (2007), respectively, we wanted to be able to detect a difference among means of 12% (mortality) and 50 ng/mL (cortisol levels) using their reported standard deviations. Our desired power level is 0.90 and our alpha level is 0.05. We used SAS version 9.1, a statistical software package published by the SAS Institute Inc., to run the power calculation. Based on this calculation the minimum sample size needed to provide the desired power level, where sample size per group = n/2, is 14.

Because fish will be marked prior to testing, we can group fish as a function of transport volume (*i.e*., all five densities of fish transported in 75% water volume will go into the same holding tank) for our post transport survival analysis. Therefore, we will require one temperature controlled tank (R1 TAF) per treatment condition (transport volume × density) per week (96 h). Assuming a minimum of four temperature controlled tanks are available for this purpose, we will be able to conduct two repetitions per week per treatment. If we successfully complete two replicates per treatment each week, it should take approximately 7−8 weeks to complete these experiments. Because the majority of this proposed research will be conducted in day one of each week, and also to maximize efficiency, we will attempt to conduct this research in combination with other TFCF research proposed by Zak Sutphin, Don Portz, and Brandon Wu.

Data Analysis

If assumptions necessary to model using parametric statistics (normality and equality of variance) are achieved, a two-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons will be used to test for differences between plasma constituent levels (Hct., lactate, glucose and cortisol) and 96 h mortality levels for controls and water volume × fish density treatment combinations. If ANOVA assumptions are not met, Kruskal-Wallis ANOVA on ranks and Dunn's test will be employed. All statistical analyses will be conducted using Sigmastat 3.0 (Jandel Scientific, San Rafael, California.) statistical software with an alpha level for all analyses set at 0.05.

Coordination and Collaboration

Experimental design and research updates will be provided at requested TTAT and/or CVFFRT meetings. However, primary coordination and collaboration will be between TFCF staff and biologists, the Fisheries and Wildlife Resources Group, SAIC government contractors, and the interagency TTAT.

Endangered Species Concerns

Fish species used in this study, threadfin shad and striped bass, are non-native to the SSJD and are not listed under the Federal or California State Endangered Species Acts (ESA). Both species will be collected at the TFCF under permitted quantities by Reclamation employees. California Scientific Collecting Permit 802025-02 has been obtained for all primary investigators and all test species.

Dissemination of Results (Deliverables and Outcomes)

Research updates will be provided and/or presented at regularly scheduled Tracy Technical Advisory Team (TTAT) and Central Valley Fish Facilities Review Team (CVFFRT) meetings. The primary deliverables will be a Tracy Volume Series, as well as a publication in a peer-reviewed scientific journal. However, Posters and/or oral presentations will also be given at appropriate scientific meetings (*i.e.,* American Fisheries Society). Additionally, information obtained in this study will be used in the implementation of new fish transportation tables for use at south SSJD fish collection facilities.

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